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The not-so-Irish spurge: *Euphorbia hyberna* (Euphorbiaceae) and the Littletonian plant “steeplechase”

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Running title: Phylogeography of *Euphorbia hyberna*

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1 The disjunct distributions of the Lusitanian flora, which are found only in southwest Ireland
2 and northern Iberia, and are generally absent from intervening regions, have been of great
3 interest to biogeographers. There has been a long debate as to whether Irish populations
4 represent relicts that survived the Last Glacial Maximum (LGM; *ca.* 21ka), or whether they
5 recolonized from southern refugia following the retreat of the ice, and if so, whether this was
6 directly, due to long distance dispersal, or successively, in the manner of a “steeplechase”,
7 with the English Channel and Irish Sea representing successive “water-jumps” that have to be
8 successfully crossed. In the present study, we used a combined palaeodistribution modelling
9 and phylogeographical approach to elucidate the glacial history of the Irish spurge,
10 *Euphorbia hyberna*, the sole member of the Lusitanian flora that is also thought to occur
11 naturally in southwestern England. Our findings suggest that the species persisted through
12 the LGM in several southern refugia, and that northern populations are the result of
13 successive recolonization of Britain and Ireland during the postglacial Littletonian warm
14 stage, akin to the “steeplechase” hypothesis.

15
16 **ADDITIONAL KEYWORDS:** *Euphorbia hyberna* – Irish spurge – Last Glacial Maximum –
17 Lusitanian flora – palaeodistribution modelling – phylogeography

INTRODUCTION

The present-day distributions of many species are the result of climatic fluctuations during the Quaternary period (*ca.* 2.5 MYA – present; Webb & Bartlein, 1992; Comes & Kadereit, 1998; Hewitt, 2003; Provan & Bennett, 2008). During the most recent (Weichselian) glacial period in Europe (*ca.* 115 KYA – 20 KYA), these species persisted in refugia associated with the southern peninsulas of Iberia, Italy and the Balkans, although fossil and genetic data also indicate survival in more northerly, or “cryptic”, refugia (Taberlet *et al.*, 1998; Hewitt, 1999; Stewart & Lister, 2001; Bennett & Provan, 2008; Provan & Bennett, 2008). Phylogeographic analyses in particular have allowed the reconstruction of various patterns of postglacial recolonization from one or more of these refugia that have led to current-day species distributions (Taberlet *et al.*, 1998; Petit *et al.*, 2003).

Ireland represents a particularly interesting biogeographical case-study in postglacial recolonization. As an island on the western fringe of the European continent, it has been isolated from Britain by postglacial sea-rise for *ca.* 15 KY, twice as long as Britain itself has been isolated from mainland Europe (Edwards & Brooks, 2008). Ireland has a relatively impoverished flora, having only *ca.* 800 species compared to nearly 1,200 in Britain and 3,500 in France, and with only 18 of these not found in Britain compared to 375 British species absent from Ireland (Reid, 1913; Webb, 1983). This has been attributed to the recolonization of Britain and Ireland during the postglacial Littletonian warm stage being somewhat akin to a “steepchase”, with the English Channel and Irish Sea representing successive “water-jumps” that have to be successfully crossed (Mitchell & Ryan 1992; Jones, 2011).

The so-called “Lusitanian” element of the Irish flora comprises a number of species that exhibit a disjunct distribution, being found in southern and western Ireland, as well as in

northern Spain, but mostly absent from intervening countries (Matthews, 1926; Praeger, 1933, 1939; Baker, 1959; Webb, 1983). The Lusitanian distribution has been the subject of debate for many years, with some botanists claiming it to be the result of persistence in separate Irish and Iberian refugia during the last glaciation (Forbes, 1846; Praeger, 1933), whilst others favoured long-distance dispersal from a southern refugium, claiming that full glacial conditions precluded *in situ* survival in Ireland (Reid, 1913). Recently, the first phylogeographic studies on three plant species exhibiting Lusitanian distributions, *Daboecia cantabrica*, *Pinguicula grandiflora* and *Saxifraga spathularis*, concur with the latter hypothesis and suggest that they achieved their current distributions as a result of recolonization from a range of refugia in Iberia and the Bay of Biscay following the last glacial maximum (LGM; *ca.* 21 ka; Beatty & Provan, 2013, 2014).

Irish spurge (*Euphorbia hyberna*) is the sole member of the Lusitanian flora that also occurs naturally, although very sporadically, in southwestern England (Devon and Cornwall) and in central and southern France, as well as in northern Spain and southwestern Ireland (Figure 1a). Unlike the previously studied Lusitanian plant species named above, which have minute, dust-like seeds conducive to long-distance dispersal, *E. hyberna* has large (3-5 mm) seeds with far less capacity for dispersal. Consequently, given its occurrence in the intervening countries between Ireland and Spain, we employed a combined palaeodistribution modelling and phylogeographical approach to test whether the species persisted during the LGM in northern refugia, or whether the colonization of Ireland could have taken place in the sequential fashion of the Littletonian plant “steepchase”. We analysed the distribution of genotypes at one chloroplast and one nuclear marker from samples across the species’ range in combination with the palaeodistribution model to identify the locations of glacial refugia during the LGM, and to elucidate how postglacial recolonization has resulted in the species’ current-day distribution.

MATERIALS AND METHODS

STUDY SPECIES

Euphorbia hyberna is a monoecious perennial which can be found growing in woodland glades, hedgerows and on shaded stream banks, and is most easily recognized from April to June when it flowers. The yellow-green flowers are formed on tall stems which can be up to 60 cm tall, and lack petals and sepals. The sap is poisonous, containing phorbol esters, and can cause irritation to the skin. In England, the species is classed by the IUCN as “Vulnerable”, based on data from The Vascular Plant Red Data List (IUCN, 2001).

SAMPLING AND DNA EXTRACTION

Samples of *E. hyberna* were collected from six locations across the species’ Irish range in Counties Cork and Kerry. Leaf samples were also obtained from herbarium specimens from a further 19 locations in Ireland, three locations in England, eight locations from France, and from 36 locations spanning the species’ entire Spanish distribution (see Fig.1 and Supporting Information Table S1 for details of locations and numbers of samples). DNA was extracted from field-collected material using a modified CTAB (cetyl trimethyl ammonium bromide) protocol (Doyle & Doyle, 1987) and from herbarium samples using Qiagen DNeasy kits.

PALAEODISTRIBUTION MODELLING

We modelled suitable climate envelopes for *E. hyberna* at the LGM (*ca.* 21 ka) in order to predict potential refugia, using an ensemble modelling (EM) approach (R Biomod2 package; Thuiller *et al.*, 2012). Species occurrence data (412 spatially unique records; Fig.2a) were obtained from the Global Biodiversity Information Facility data portal (<http://www.gbif.org/>). Ten different distribution models using as explanatory covariates the first four principle

component (PC) scores from 19 BIOCLIM variables (WorldClim data set 1950–2000; Hijmans *et al.*, 2005) were generated at 2.5 arc-minute resolution extracted from an area bounded by 15°W to 30°E and 35°N to 60°N. These first four principal components accounted for 99% of the variation in the climate data. We used this approach because using the BIOCLIM variables directly failed even when selecting from the 19 variables by removing the most strongly correlated ones ($r > 0.7$). While the current-climate models looked plausible and had good AUC (area under the curve) of the ROC (Receiver-Operator Characteristic) scores ($AUC > 0.8$), the resultant palaeoclimatic projections were very different from each other; this was traced to instability caused by the strong cross correlations still present between the explanatory variables (Dormann *et al.*, 2013). The ten models were screened for performance using the conventional 70/30 training/validation data partition. Each model was run ten times using this partition. Eight models passed this filter (FDA, flexible discriminant analysis, did not converge and we excluded SRE, surface range envelope, because it had a consistently lower AUC goodness-of-fit measure (Supporting Information Table S2). These remaining eight models (ten replicates of each) were then combined using the ROC measure to give an EM, using the median measure, and propagating the uncertainty from the training/test split from the 80 fitted models. The rationale for an EM approach is that such a composite model often outperforms individual models (Seni & Elder, 2010), though it is by no means settled how best this can be done for species distribution modelling. This EM was then supplied with reconstructed LGM data [Community Climate System Model (CCSM); Palaeoclimate Modelling Intercomparison Project Phase II: <http://pmip2.lsce.ipsl.fr/>] to identify potential *E. hyberna* refugial areas. We calculated a multivariate environmental similarity surface (MESS; Elith *et al.*, 2010) to ensure that the species range projected at the LGM climate was not outside the current climate – in other words, that the LGM projections were not extrapolations outside current climate space.

CHLOROPLAST *trnS-trnG* SEQUENCING

In total, 200 samples were sequenced for the chloroplast *trnS-trnG* intergenic spacer. A product was initially amplified and sequenced using the universal *trnS-trnG* primers described in Zhang *et al.*, (2005), and a pair of species-specific primers were subsequently designed: Eh-*trnS* 5'-CATCTCTCCCGATTGAAAAGG-3' and Eh-*trnG* 5'-TAAACTATACCCGCTACGATACAA-3'. For herbarium samples from which the complete product could not be amplified in a single polymerase chain reaction (PCR), the region was amplified as two or three overlapping fragments using the primers described in Supporting Information Table S3. PCR was carried out on a MWG Primus thermal cycler (Ebersberg, Germany) using the following parameters: initial denaturation at 94 °C for 3 min followed by 45 cycles of denaturation at 94 °C for 30 s, annealing at 58 °C for 30 s, extension at 72 °C for 1 min and a final extension at 72 °C for 5 min. PCR was carried out in a total volume of 20 µL containing 200 ng genomic DNA, 10 pmol of each primer, 1× PCR reaction buffer, 200 µM each dNTP, 2.5 mM MgCl₂ and 0.5 U GoTaq Flexi DNA polymerase (Promega, Sunnyvale CA). 5 µL volumes of PCR products were resolved on 1.5% agarose gels and visualized by ethidium bromide staining, and the remaining 15 µL were ExoSAP-purified and sequenced in both directions using the BigDye sequencing kit (v3.1; Applied Biosystems, Foster City, CA) and run on an AB 3730XL DNA analyser (Life Technologies; Carlsbad, CA). Sequence lengths ranged from 548 bp – 563 bp, and the overall alignment was 628 bp in length.

SINGLE-COPY NUCLEAR DNA (SCNDNA) SEQUENCING

Primers to amplify an anonymous single-copy nuclear DNA locus (Eh-E04) were developed using the ISSR cloning method described in Beatty, Philipp & Provan (2010). The 214 bp region was amplified in 200 individuals using the following primers: Eh-E04-F

5'-TTCCAAATTCCAATTCTGTGC-3' and Eh-E04-R

5'-CATCATCATTCAATTAACAAATAAA-3. PCR and sequencing were carried out as described above.

PHYLOGEOGRAPHIC ANALYSIS

For the *scnDNA* locus, potential recombination was assessed using the Hudson & Kaplan (1985) test in the DnaSP software package (V5.10; Librado & Rozas 2009). As no evidence of recombination was detected, haplotypes were resolved for individuals exhibiting two or more heterozygous positions using the PHASE program (V2.1; Stephens & Donnelly 2003) implemented in DnaSP. DNA sequences were aligned in BIOEDIT (V7.0.9.0; Hall, 1999). Median-joining networks for both regions were constructed using the NETWORK software package (V4.5.1.6; www.fluxus-engineering.com). Any reticulations in the networks were broken following the rules described in Pfenninger & Posada (2002).

To identify groups of populations in Spain and France associated with potential refugial areas, we performed a spatial analysis of molecular variance (SAMOVA) using the software package SAMOVA (V1.0; Doupanloup, Schneider & Excoffier, 2002) for both of the data sets. This program uses a simulated annealing approach based on genetic and geographical data to identify groups of related populations. The program was run for 10,000 iterations for $K = 2$ to 10 groups, from 200 initial conditions, and the most likely structure was identified using the maximum value of Φ_{CT} , the proportion of genetic variation between groups of populations, that did not include any groups of a single population.

Levels of haplotype diversity (H) and nucleotide diversity (π) at both the chloroplast and *scnDNA* locus were calculated for mainland European, English and Irish samples using DnaSP. To account for differences in sample sizes, effective numbers of haplotypes (h_e) were also calculated using HAPLOTYPE ANALYSIS 1.05 (Eliades & Eliades, 2009).

RESULTS

PALAEODISTRIBUTION MODELLING

The current climate species distribution ensemble model (Fig.2) and its component eight distribution models based on current climate (Supporting Information Fig. S1) have what is usually considered to be high AUC values (Supporting Information Table S2; EM AUC=0.974) and, as is often the case, good visual congruence between current distribution records and areas of suitable climate (except the individual MARS model). Example model projections for the component eight models for the LGM are given in Supporting Information Fig. S2, and the climatic data (PC scores) used are mapped in Supporting Information Figs. S3 and S4. Notable in Fig.2 is the general spread of climatically suitable areas beyond the current *Euphorbia hyberna* range, such as north western Ireland, western Britain and Belgium. There are also a few *Euphorbia hyberna* records in areas of apparently low current climatic suitability, such as northern Italy and Slovenia. In contrast to the current EM, the paleodistribution EM is spatially rather more coherent in that there is a wide band of suitable climate extending from northern Spain and southwest France across the Bay of Biscay and up to the south and west of Ireland and the LGM ice sheet (Fig. 2). Within this range of suitable past climate there are areas identified by the EM as particularly suitable and so more likely as potential refugia. These include in Spain the four main areas of Castile and León, northern Galicia, northern Aragon and Cataluña, and in France a small area on the island of Corsica, a large area centered on the Poitou Charentes region, and a smaller one in the Maritime Alps. Finally, there are three suitable regions in the area now covered by the Atlantic: in the Bay of Biscay at the western tip of Brittany, larger area to the south of this, and an isolated area well to the south of Ireland. The multivariate environmental similarity surface (MESS; Supporting Information Fig. S5) indicated that only a small amount of

extrapolation into novel climate space occurred, and that this was primarily in the north where the ice sheet was present.

PHYLOGEOGRAPHIC ANALYSIS

18 and 14 haplotypes were identified for the chloroplast *trnS-trnG* intergenic spacer and the anonymous Eh-E04 single copy nuclear locus, respectively (Figs. 3a/b; GenBank accession numbers XXX – XXX and XXX – XXX). The SAMOVA analyses based on the *scnDNA* locus did not identify more than a single group comprised of more than one individual, but the analysis based on the chloroplast *trnS-trnG* region identified $K = 4$ groups ($\Phi_{CT} = 0.728$; Fig. 1). Some degree of geographical structuring of haplotypes was evident for both loci, but particularly for the chloroplast *trnS-trnG* intergenic spacer (Fig. 3a). The dark blue, light blue, pink and white haplotypes were restricted to the eastern part of the main continental distribution of *E. hyberna*, around the Pyrenees, whilst the yellow, light yellow, brown and purple haplotypes were only found west of this region. This was broadly reflected in the distributions of the haplotypes displayed by the anonymous Eh-E04 single copy nuclear locus (Fig. 3b). For both loci, the distribution of the green haplotype was of particular note. This haplotype was found in England, along with the ubiquitous red haplotype, but in mainland Europe was restricted to a single location in the extreme northeast of the Basque Country in Spain, adjacent to the Bay of Biscay. All diversity statistics for the two markers indicate a decrease in levels of genetic diversity from mainland Europe, through England, to Ireland, where both loci were monomorphic (Table 1).

DISCUSSION

The EM for the current climate and distribution (Fig. 2) suggests that *Euphorbia hyberna* is not climatically limited with respect to future expansion into much of Britain and Ireland. Belgium is another notably climatically suitable but unoccupied area. These unoccupied but apparently suitable areas serve as a reminder that apparently suitable locations need not be occupied, since species have requirements and traits other than climate that must be present for effective recolonization and population persistence. *E. hyberna* has large, globose seeds, with no obvious modifications to aid dispersal, and absence from climatically suitable regions may occur due to low capacity for dispersal, or simply because its other niche requirements, such as suitable substrate and/or nutrients, are not met. Alternatively, such areas may be attributable to the modelling approach, despite the high AUC scores. In general, it is becoming apparent that model uncertainty is not well dealt with by current species distribution models, including those used here, particularly uncertainty caused by structural model misspecification (e.g. ignoring autocorrelation in explanatory covariates), response variable quality (e.g. variation in spatial recording effort), and low-quality predictive covariate data or predictive covariate data that represents an extrapolation (Beale & Lennon, 2012). For this reason the paleodistribution model results here, and elsewhere, are best taken as a broadly indicative rather than as definitive of potential refugia locations, despite the high AUC score (Beale, Lennon & Gimona, 2008).

The paleodistribution model identified a number of potential refugia at the LGM, several of which overlap the current distribution of *E. hyberna*. These areas are comparable in size and climatic suitability (by definition) to the current distribution. The ensemble model is of nominally good fit but of course there is no way to establish from modelling alone if any particular putative refugium was occupied at the LGM; several may have been unoccupied as

a consequence of unsatisfied non-climatic niche requirements. However, based on a combination of this palaeodistribution modelling and the spatial structuring observed in genetic data from two independent loci (particularly the chloroplast data) it appears that the species persisted in multiple southern refugia during the glaciations. The findings of the present study further highlight the complex nature of refugial persistence in the Iberian Peninsula. The previous concept that each of the three southern peninsulas of Iberia, Italy and the Balkans represented a single refugial area has now been superseded by the idea of “refugia-within-refugia”, originally proposed for Iberia, but now with several examples from the other peninsulas (Gomez and Lunt, 2007). The potential persistence of *E. hyberna* in separate eastern and western refugia, as suggested by the genetic data and to some extent the palaeodistribution model, which indicated an area of suitable habitat in Galicia, mirrors the patterns found for *D. cantabrica* (Beatty & Provan, 2013). The existence of two SAMOVA groups (shown in blue and pink in Fig. 1) apparently associated with the Pyrenees could be explained by divergence of these groups in separate microrefugia / microhabitats within, or in close proximity to, this region. A previous phylogeographical study on white oaks (*Quercus* spp.) in Iberia also identified two lineages (designated Lineage A and Lineage C) restricted to the eastern Pyrenees that were hypothesized to have originated in separate Cataluñan refugia (Olalde *et al.*, 2002). *E. hyberna* is most commonly found in oak woodland, and it is possible that the two taxa, *E. hyberna* and *Quercus* spp., could have persisted together during the LGM. The general west / east distribution of the pink and blue groups respectively along the Pyrenees, could indicate persistence in separate microrefugia in the Pyrenees. Such refugia could be difficult to identify using the palaeodistribution model at the resolution used, particularly given that mountainous regions can provide a range of spatial and elevational habitats that could conceivably promote divergence of lineages (Bennett & Provan, 2008; Médail & Diadema, 2009; Holderegger & Thiel-Egenter, 2009; Stewart *et al.*, 2010).

Our findings strongly suggest that the present disjunct distribution of *E. hyberna* has resulted from recolonization of England and Ireland from a southern refugium, possibly in the region of the Bay of Biscay (see below). A similar scenario for the postglacial recolonization of Ireland was found in the two previous phylogeographic studies on plants exhibiting Lusitanian distributions (Beatty & Provan, 2013, 2014). In general, Irish populations exhibited lower levels of genetic diversity than those in Spain, with *E. hyberna* completely lacking any private haplotypes, classic signatures of postglacial recolonization rather than glacial persistence (Provan & Bennett, 2008). Thus, although the palaeodistribution model indicated possible suitable habitat for *E. hyberna* along the edge of the continental shelf as far as the limits of the British-Irish ice sheet at the LGM, the phylogeographic evidence indicates that if such a refugium did exist on land that is now submerged, and was the source of the northern populations, it must have been situated much further south.

The fact that *E. hyberna* is the only plant with a Lusitanian distribution that also occurs naturally in England means that alternative theories on the postglacial recolonization of Lusitanian species can be examined. In the previous phylogeographic studies on elements of the Lusitanian flora, the extremely disjunct distribution of present-day populations meant that it was not possible to differentiate between recolonization as a result of long-distance dispersal, and a more gradual “stepping-stone” pattern of recolonization followed by subsequent extirpation of the species from intervening areas (Beatty & Provan, 2013, 2014). The sequential decrease in genetic diversity observed in continental, English and Irish populations of *E. hyberna* in both markers studied is entirely consistent with the “steeplechase” scenario following the end of the Weichselian glaciation (Matthews, 1926; Mitchell & Ryan 1992; Jones, 2011). At both loci, the haplotypes indicated in green, which in Spain are restricted to populations in the extreme north, close to the French border and adjacent to the Bay of Biscay, are found along with the most common red haplotype in

England, but only the latter is present in Ireland. Our evidence that *E. hyberna* colonized Ireland in the fashion of the Littletonian plant “steepchase” thus raises the intriguing possibility that other Lusitanian species might have achieved their present-day distributions in a similar manner, with no need to invoke extreme long-distance dispersal events. A similar scenario was originally proposed nearly 100 years ago based on biogeographical data (Stapf, 1914, 1916), but the present study represents the first test of this hypothesis at the intraspecific level (although see Valtueña, Preston & Kadereit, 2012 for a species with a similarly disjunct distribution, despite not being strictly a Lusitanian one).

There also remains the possibility that the present disjunct distribution of *E. hyberna* (and other members of the Lusitanian flora) might be due to anthropogenic transport. The extremely low levels of genetic variation in Irish populations could be consistent with a single, recent introduction, and there are many documented cases of the introduction, deliberately or accidentally, of mammals into Ireland by humans since Mesolithic times (reviewed in Montgomery *et al.* 2014). The role of humans in the introduction of the Lusitanian flora, however, has rarely been considered. With the exception of the Strawberry tree, *Arbutus unedo*, which has been planted as an ornamental, botanists generally now believe that anthropogenic introduction of these species to Ireland is unlikely, and that natural postglacial recolonization is the best explanation for their present-day distributions (Sealy 1949). Although no macrofossil or palynological evidence exists for *E. hyberna* in Ireland, at least one other member of the Lusitanian flora, *Daboecia cantabrica*, is known to have been present as far back as the Gortian (Holsteinian) interglacial (*ca.* 428-302 ka; Woodell 1958; Coxon 1996).

In conclusion, our findings suggest that the present-day disjunct distribution of *Euphorbia hyberna* did not result from glacial survival in separate northern and Iberian refugia, contrary to Forbes’ (1846) original idea on the origin of the Lusitanian distribution, but instead is due

314 to the recolonization of England and Ireland from a southern refugium, possibly in the area
315 around the Bay of Biscay as also previously indicated for *Daboecia cantabrica*. Whilst these
316 results cast doubt on one early biogeographical hypothesis on the origin of the Lusitanian
317 flora, they do provide support for another, namely Stapf's (1914, 1916) theory of progressive
318 dispersal in the fashion of the Littletonian "steeplechase". Furthermore, they highlight the
319 complex processes responsible for the present-day distribution of genetic variation in the
320 Iberian Peninsula, as well as those operating at species' rear-edges in general, where
321 populations often represent reservoirs of unique genetic diversity (Hampe & Petit, 2005;
322 Provan & Maggs 2012).

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Table 1. Diversity statistics by region.

| Locus | Mainland Europe | | | | | England | | | | | Ireland | | | | | GenBank |
|------------------|------------------|----------|----------------------|----------|--------|-----------------|----------|----------------------|----------|--------|------------------|----------|----------------------|----------|-------|------------|
| | <i>N</i> | <i>h</i> | <i>h_e</i> | <i>H</i> | π | <i>N</i> | <i>h</i> | <i>h_e</i> | <i>H</i> | π | <i>N</i> | <i>h</i> | <i>h_e</i> | <i>H</i> | π | Accessions |
| <i>trnS-trnG</i> | 114 | 18 | 3.672 | 0.782 | 0.0032 | 10 | 2 | 1.724 | 0.467 | 0.0009 | 76 | 1 | 1 | - | - | |
| Eh-E04 | 234 ^a | 14 | 1.377 | 0.289 | 0.0015 | 20 ^a | 2 | 1.220 | 0.189 | 0.0009 | 146 ^a | 1 | 1 | - | - | |

^a Two gene copies sequenced per diploid individual

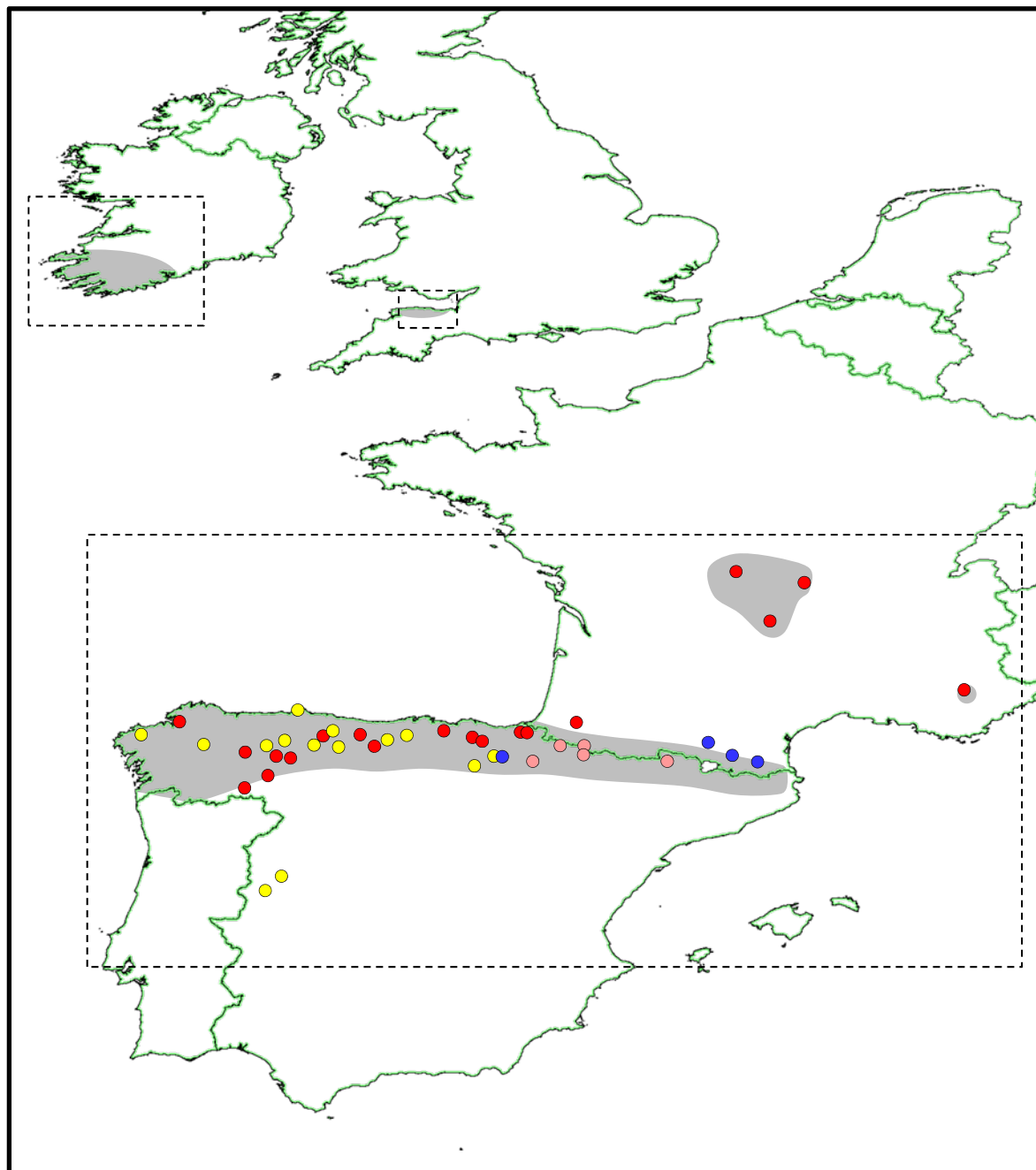
Abbreviations: *N*, number of individuals studied; *h*, number of haplotypes; *h_e*, effective number of haplotypes; *H*, gene diversity; π , nucleotide diversity.

Figure Legends

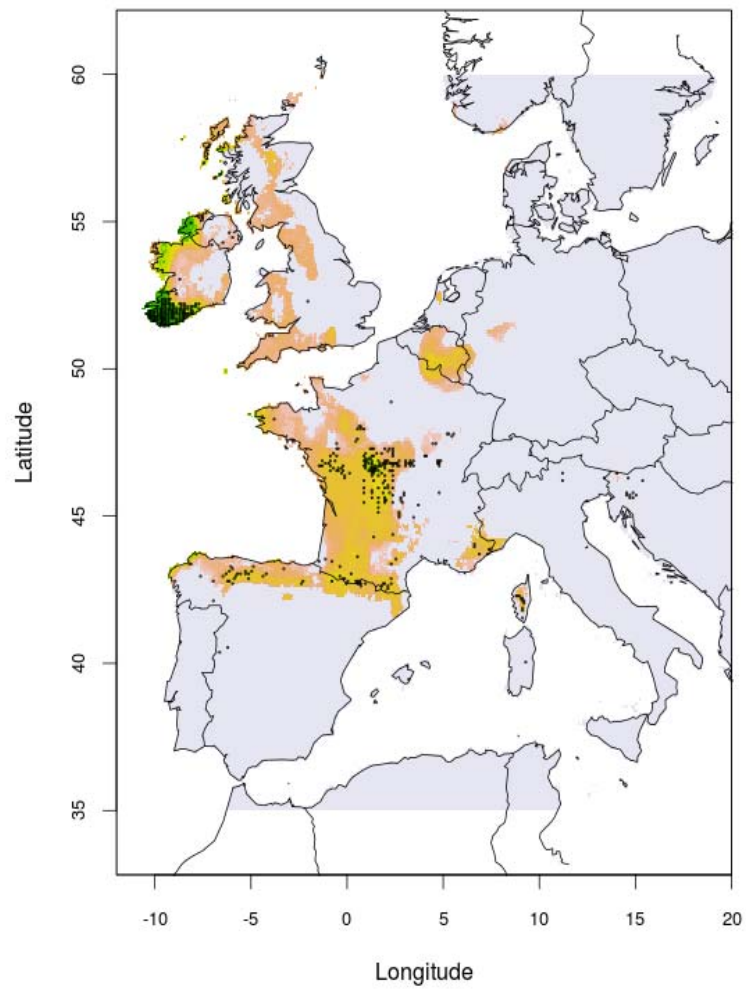
Fig. 1. Present-day distribution of *Euphorbia hyberna* [shaded; based on Webb, 1983, and the Global Biodiversity Information Facility (data.gbif.org)] in Western Europe. Dashed areas show the regions highlighted in Fig. 3a/b. Coloured circles depict assignment of populations to one of $K=4$ clusters by the SAMOVA analysis.

Fig. 2. Modelled distribution of *Euphorbia hyberna* (a) current climate and (b) at the Last Glacial Maximum, (LGM, *ca.* 21 ka). The limits of the ice sheets (after Sejrup *et al.*, 2005) at the LGM are also indicated. Both panels show the modelled species range according to an ensemble model based on ten replicates of eight different model types as the coloured areas. Within this range there is variation in climatic suitability, as indicated by the colour shading.

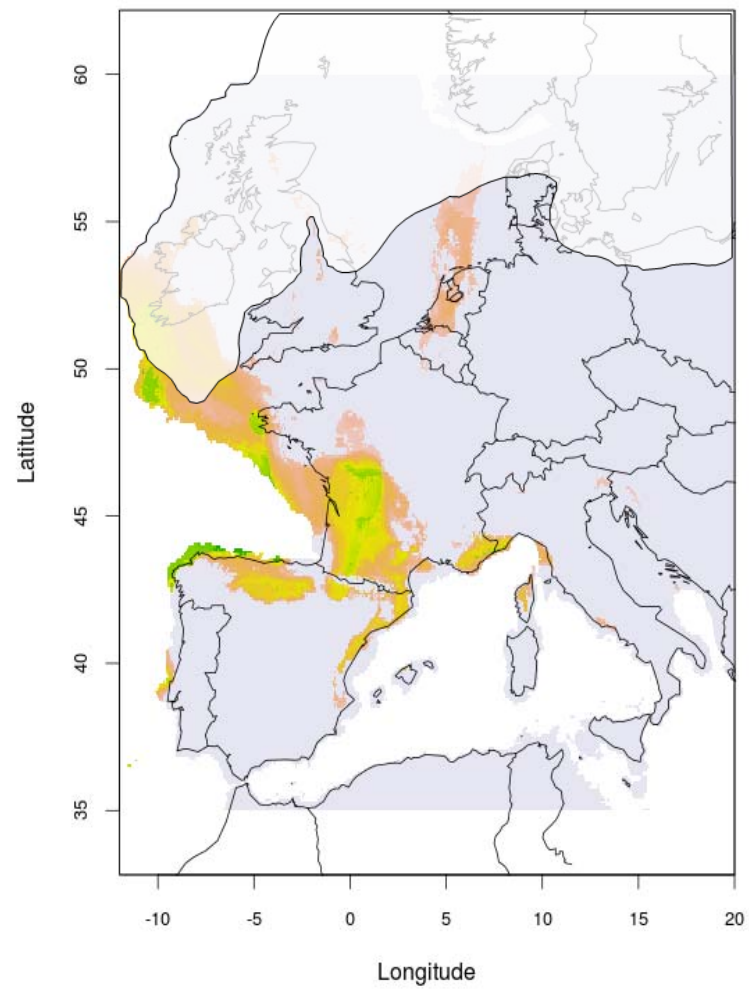
Fig. 3. Haplotype distributions for (a) chloroplast *trnS-trnG* region and (b) nuclear Eh-E04 region for *Euphorbia hyberna* in Western Europe. Pie chart sizes are approximately proportional to sample size, with the smallest circles representing $N=1$ (chloroplast locus) or $N=2$ (nuclear locus) and the largest representing $N=8$ (chloroplast locus) or $N=16$ (nuclear locus). In the haplotype networks, circle sizes are approximately proportional to haplotype frequency. Open diamonds represent missing haplotypes and small black circles represent unique haplotypes i.e. those found in a single individual. Codes for single-individual haplotypes refer to IH codes and barcodes given in Supplementary Information Table S1.



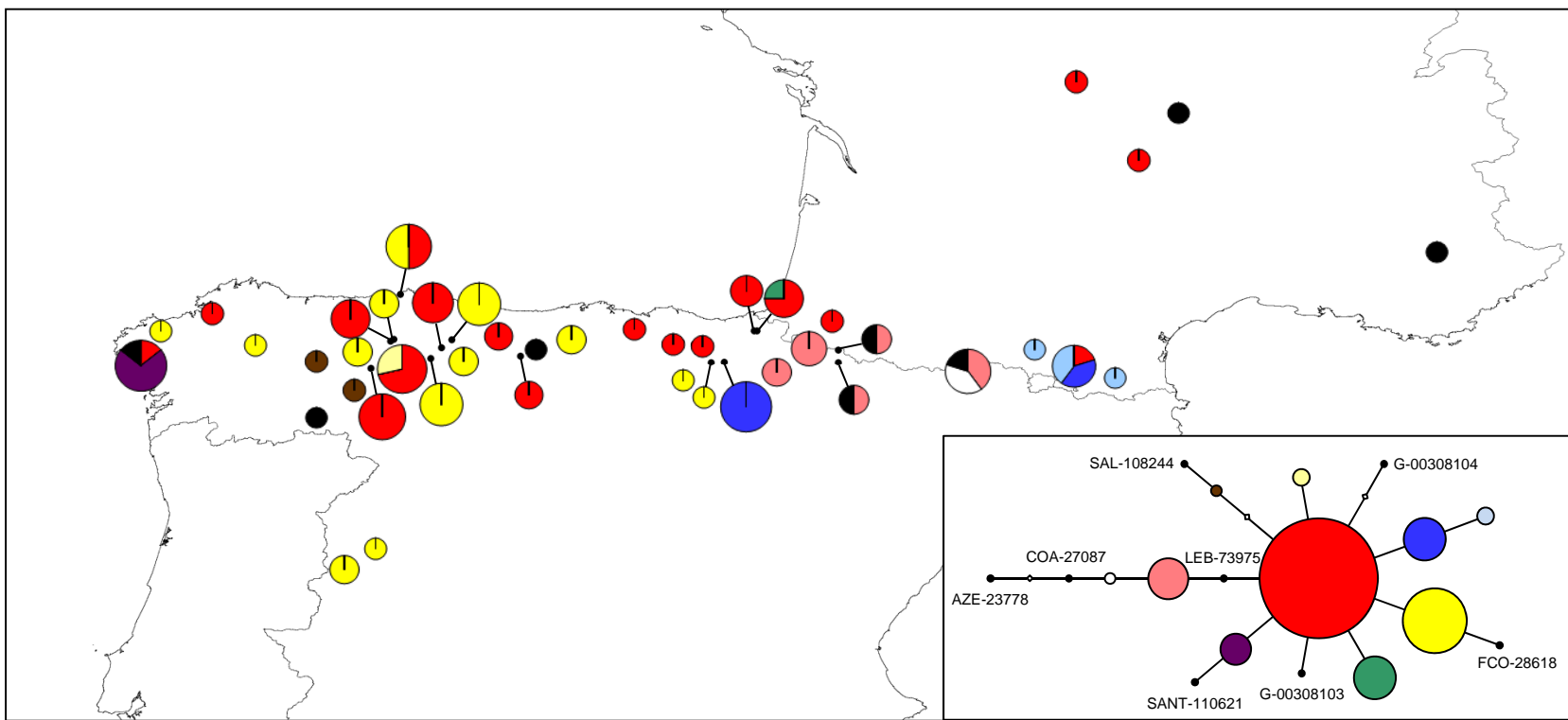
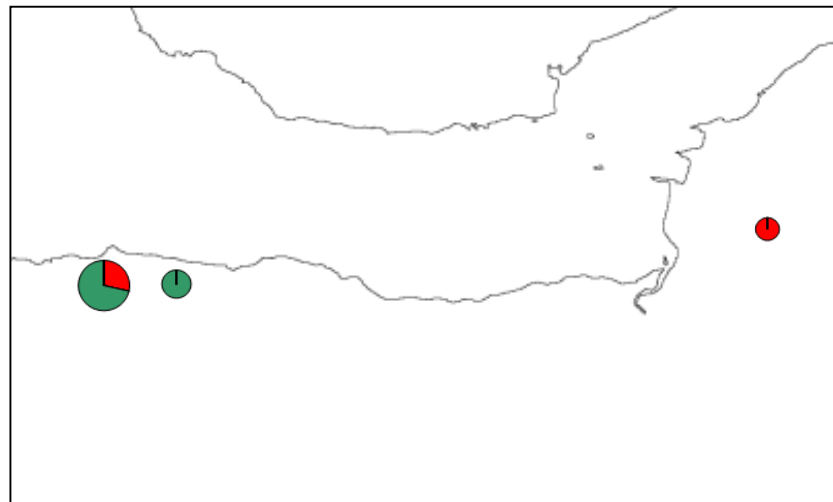
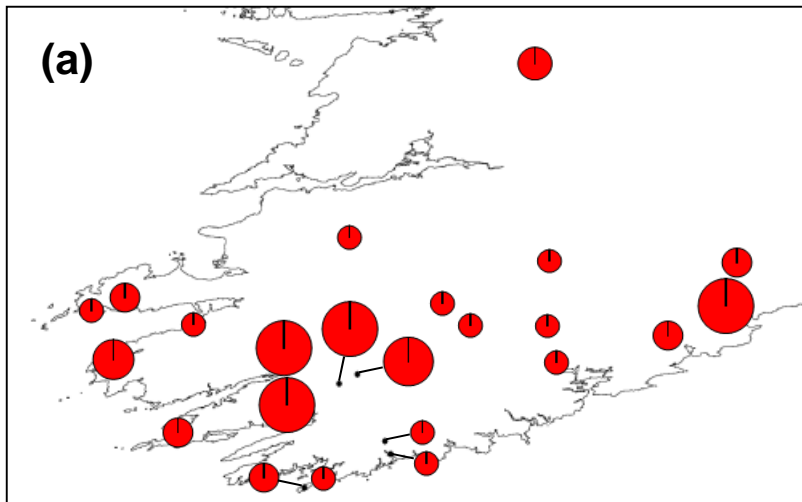
Current climate model



LGM climate model



(a)



(b)

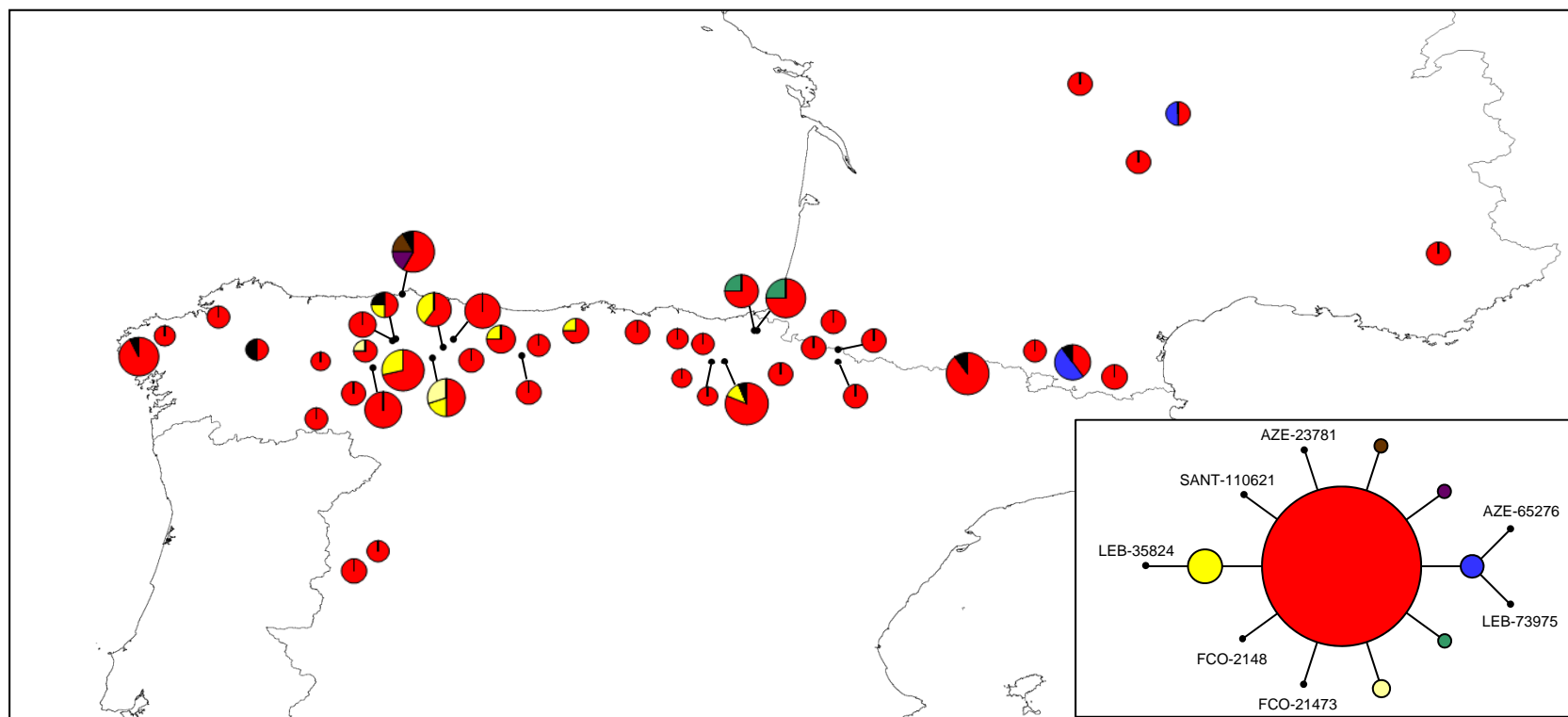
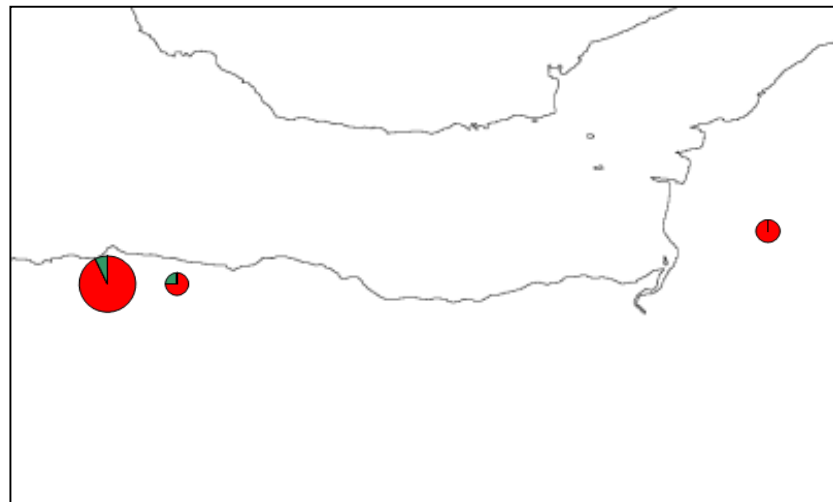
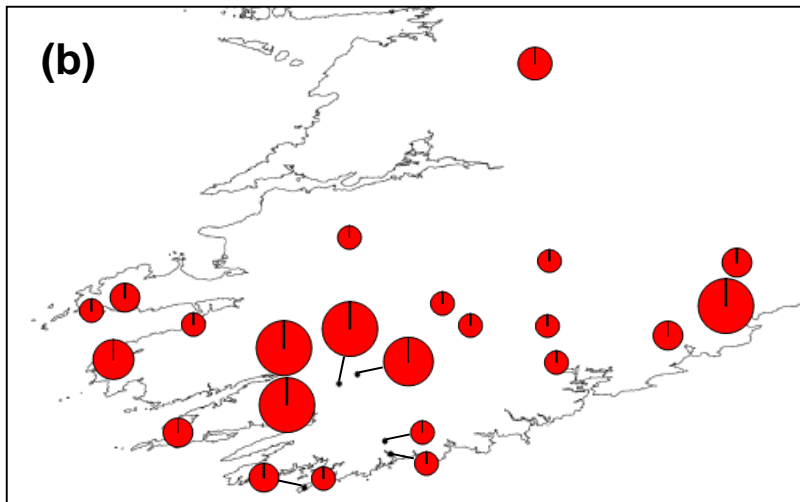


Table S1 Populations analysed in this study

| Country | Location | Code ^a | Latitude (N) | Longitude (W) | N | |
|---------|-------------------|-------------------|--------------|---------------|------------------|--------|
| | | | | | <i>trnS-trnG</i> | Eh-E04 |
| Ireland | Fahan | DBN-95-1990 | 52.106499 | -10.410695 | 1 | 1 |
| | Valentia Island | DBN-87-1980 | 51.923101 | -10.321638 | 4 | 4 |
| | Dingle | DBN-53-3790 | 52.144635 | -10.270301 | 2 | 2 |
| | Inishturk | BIRM-019143 | 53.7 | -10.1 | 1 | - |
| | Black Head | DBN-20-1961 | 51.639034 | -10.042036 | 2 | 2 |
| | Rossbeigh | DBN-22-1967 | 52.057273 | -9.974015 | 1 | 1 |
| | Derrycunihy | IRDE ^b | 51.966667 | -9.583333 | 8 | 8 |
| | Derrycunihy | IRKB ^b | 51.971333 | -9.581667 | 8 | 8 |
| | Glengarriff | IRGG ^b | 51.75 | -9.566667 | 8 | 7 |
| | Cape Clear Island | DBN-11-1964 | 51.438435 | -9.494109 | 2 | 2 |
| | Sherkin Island | DBN-55-1975 | 51.466159 | -9.417596 | 1 | 1 |
| | Gougane Barra | IRGB ^b | 51.835167 | -9.345 | 8 | 7 |
| | Alleghaun River | DBN-33-1990 | 52.387281 | -9.301819 | 1 | 1 |
| | Gurteenowen | IRGU ^b | 51.86905 | -9.265833 | 6 | 6 |
| | Drinagh | DBN-50-1980 | 51.615221 | -9.149051 | 1 | 1 |
| | Glendore | DBN-54-1972 | 51.566787 | -9.122982 | 1 | 1 |
| | Banteer | DBN-75-1977 | 52.130557 | -8.895895 | 1 | 1 |
| | Glennaharee East | DBN-102-1969 | 52.048487 | -8.780994 | 1 | 1 |
| | Derrybrien | DBN-18-1992 | 53.050328 | -8.50633 | 3 | 3 |
| | Glenville | DBN-48-1988 | 52.046378 | -8.445829 | 1 | 1 |
| | Ballyhoura Hills | DBN-104-1969 | 52.29625 | -8.438092 | 2 | 2 |
| | Glanmire | DBN-63-1976 | 51.905429 | -8.411502 | 1 | 1 |
| | Glendine | DBN-46-1964 | 52.005661 | -7.929738 | 2 | 2 |
| | Colligan Wood | IRCW ^b | 52.133333 | -7.683333 | 8 | 8 |
| | Nire Valley | DBN-23-1962 | 52.277012 | -7.634635 | 2 | 2 |

Table S1 (continued)

| Country | Location | Code | Latitude (N) | Longitude (W) | N | |
|---------|-------------------------|-------------------------------|--------------|---------------|------------------|--------|
| | | | | | <i>trnS-trnG</i> | Eh-E04 |
| England | Lynmouth | BIRM-019135/37/41/42/44/49/51 | 51.2 | -3.8 | 7 | 7 |
| | Badgworthy Water | BIRM-019147 | 51.2 | -3.7 | 2 | 2 |
| | Badgworth | BIRM-019148 | 51.265 | -2.873 | 1 | 1 |
| France | Pyrénées-Atlantiques | G-00308107 | 43.250278 | -0.879444 | 1 | 1 |
| | Ariège | G-00308108 | 42.933056 | 1.501111 | 1 | 1 |
| | Ax-les-Thermes | AZE-65276 | 42.76 | 1.96 | 5 | 5 |
| | Aubusson | G-00308109 | 46.078889 | 1.992222 | 1 | 1 |
| | Pyrénées-Orientales | G-00308110 | 42.603333 | 2.448889 | 1 | 1 |
| | Chanterelle | G-00308105 | 45.155556 | 2.716111 | 1 | 1 |
| | Puy de Dome | G-00308104 | 45.718889 | 3.186944 | 1 | 1 |
| | Alpes-de-Haute-Provence | G-00308103 | 44.090833 | 6.232222 | 1 | 1 |
| | Mazaricos | LEB-50433/SANT-110621 | 42.9389 | -8.9922 | 7 | 7 |
| Spain | Monte Casteso | LEB-31789 | 43.082449 | -8.754298 | 1 | 1 |
| | Capela | FCO-18295 | 43.35 | -8.14 | 1 | 1 |
| | Outeiro de Rei | FCO-21473 | 42.98 | -7.65 | 1 | 1 |
| | Zamora | SAL-108244 | 42.15 | -6.92 | 1 | 1 |
| | Leon | COA-27085 | 42.79 | -6.92 | 1 | 1 |
| | Caceres | SAL-64349 | 40.35 | -6.59 | 2 | 2 |
| | Palacios de Compludo | LEB-101280 | 42.452437 | -6.481113 | 1 | 1 |
| | Orallo | LEB-23973 | 42.963693 | -6.425155 | 2 | 2 |
| | Igüeña | LEB-78704 | 42.715476 | -6.282805 | 7 | 7 |
| | El Cabaco | SAL-90448 | 40.57 | -6.22 | 1 | 1 |
| | Puerto de Ventana | LEB-35824/41015 | 43.044764 | -6.053467 | 4 | 4 |
| | Oviedo | COA-6411 | 43.061419 | -6.01331 | 2 | 2 |
| | Val de Samario | LEB-60837/61455/61451 | 42.720058 | -5.967744 | 7 | 7 |

Table S1 (continued)

| Country | Location | Code | Latitude (N) | Longitude (W) | N | |
|---------|----------------------|---------------------|--------------|---------------|------------------|--------|
| | | | | | <i>trnS-trnG</i> | Eh-E04 |
| Spain | Gozón | FCO-02148/LEB-21590 | 43.59 | -5.94 | 6 | 6 |
| | Llombera | LEB-54527/54563 | 42.837471 | -5.58183 | 5 | 5 |
| | Redipuestas | LEB-16558/17188 | 42.966384 | -5.452345 | 5 | 5 |
| | Puebla de Lillo | LEB-37591/44012 | 43.058921 | -5.333235 | 5 | 5 |
| | Valdore | LEB-14420/14428 | 42.791403 | -5.200889 | 2 | 2 |
| | Llanaves de la Reina | LEB-61452 | 43.059661 | -4.792943 | 2 | 2 |
| | Ruesga | LEB-41416 | 42.865368 | -4.530235 | 2 | 2 |
| | San Cebrián de Mudá | FCO-28618 | 42.92 | -4.35 | 1 | 1 |
| | Zeanuri | AZE-5283 | 43.048 | -3.927 | 2 | 2 |
| | Balmaseda | AZE-5284 | 43.177 | -3.203 | 1 | 1 |
| | Otxandio | AZE-5282 | 43.033 | -2.65 | 1 | 1 |
| | Lagrán | AZE-5280 | 42.61 | -2.628 | 1 | 1 |
| | Aretxabaleta | AZE-5285 | 43.005 | -2.491 | 1 | 1 |
| | Alava | COA-27906 | 42.792351 | -2.290801 | 1 | 1 |
| | Améscoa Baja | AZE-23777/23781 | 42.796 | -2.138 | 8 | 8 |
| | Goizueta | AZE-23784 | 43.162 | -1.788 | 4 | 4 |
| | Aranaz | AZE-23783 | 43.162 | -1.751 | 3 | 3 |
| | Leoz | AZE-23780 | 42.664 | -1.517 | 2 | 2 |
| | Ochagavía | AZE-23782 | 42.964 | -1.142 | 3 | 6 |
| | Huesca | COA-27087 | 42.79 | -0.8 | 2 | 2 |
| | Izaba | AZE-23778 | 42.94 | -0.8 | 2 | 2 |
| | Artiga de Lin | LEB-73975/COA-31229 | 42.681502 | 0.705355 | 5 | 5 |

^a IH codes: DBN - National Botanic Gardens of Ireland, Glasnevin; BIRM - University of Birmingham Herbarium; G – University of Geneva Herbarium; SANT - Universidad de Santiago de Compostela Herbario; FCO - Universidad de Oviedo Herbario; LEB - Universidad de León Herbario; COA - Universidad de Córdoba Herbario; SAL - Universidad de Salamanca Herbario; AZE - Alto do Zorroaga s.n. Herbario.

^b These codes refer to our own, field collected samples

Table S2 AUC values from nine model types fitted using 70/30 training/test split showing ten replicates of each model. SRE was dropped and the remaining eight models used to generate an ensemble model, where the contribution model of each model to the ensemble is weighted by the AUC score.

| Model* | Run | | | | | | | | | |
|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
| GLM | 0.918 | 0.913 | 0.907 | 0.894 | 0.878 | 0.916 | 0.914 | 0.891 | 0.924 | 0.920 |
| GAM | 0.944 | 0.934 | 0.937 | 0.929 | 0.925 | 0.950 | 0.934 | 0.934 | 0.945 | 0.951 |
| ANN | 0.897 | 0.893 | 0.886 | 0.881 | 0.871 | 0.924 | 0.875 | 0.868 | 0.927 | 0.898 |
| SRE | 0.802 | 0.797 | 0.792 | 0.761 | 0.774 | 0.785 | 0.776 | 0.788 | 0.796 | 0.757 |
| CTA | 0.942 | 0.927 | 0.920 | 0.895 | 0.908 | 0.928 | 0.916 | 0.895 | 0.947 | 0.927 |
| GBM | 0.952 | 0.939 | 0.948 | 0.932 | 0.934 | 0.956 | 0.940 | 0.935 | 0.961 | 0.958 |
| RF | 0.866 | 0.831 | 0.859 | 0.845 | 0.858 | 0.834 | 0.839 | 0.834 | 0.847 | 0.867 |
| MARS | 0.913 | 0.874 | 0.897 | 0.845 | 0.846 | 0.902 | 0.932 | 0.839 | 0.902 | 0.885 |
| MaxEnt | 0.957 | 0.940 | 0.951 | 0.944 | 0.947 | 0.961 | 0.954 | 0.955 | 0.965 | 0.963 |

* GLM=Generalized Linear Model, GAM=Generalized Additive Model, ANN=Artificial Neural Network, SRE=Surface Range Envelope, CTA=Classification Tree

Analysis, GBM=Generalised Boosted Model, RF=Random Forests, MARS=Multiple Adaptive Regression Splines, MaxEnt=Maximum Entropy

Table S3 Internal primers used for sequencing chloroplast *trnS-trnG* region

| Primer | Sequence |
|------------|--------------------------------------|
| trnS-IN-R | CAATTTTTTATATTCTATTATTATATAGAATTATAG |
| trnS-IN-F | CTATAATTCTATATAATAATAGAATATAAAAAATTG |
| trnS-IN-R1 | AGAATTCTAAATATAATAGAATTAATAAAT |
| trnS-IN-F1 | CGATATTTATTAATTCTATATTTAGAATTCT |
| trnS-IN-R2 | AGTTATTAAGTTAACTATTTATTTCTATTTG |
| trnS-IN-F2 | AGAATTGAAAATTGAAATATATAGAAATTCA |

Supplementary Figures

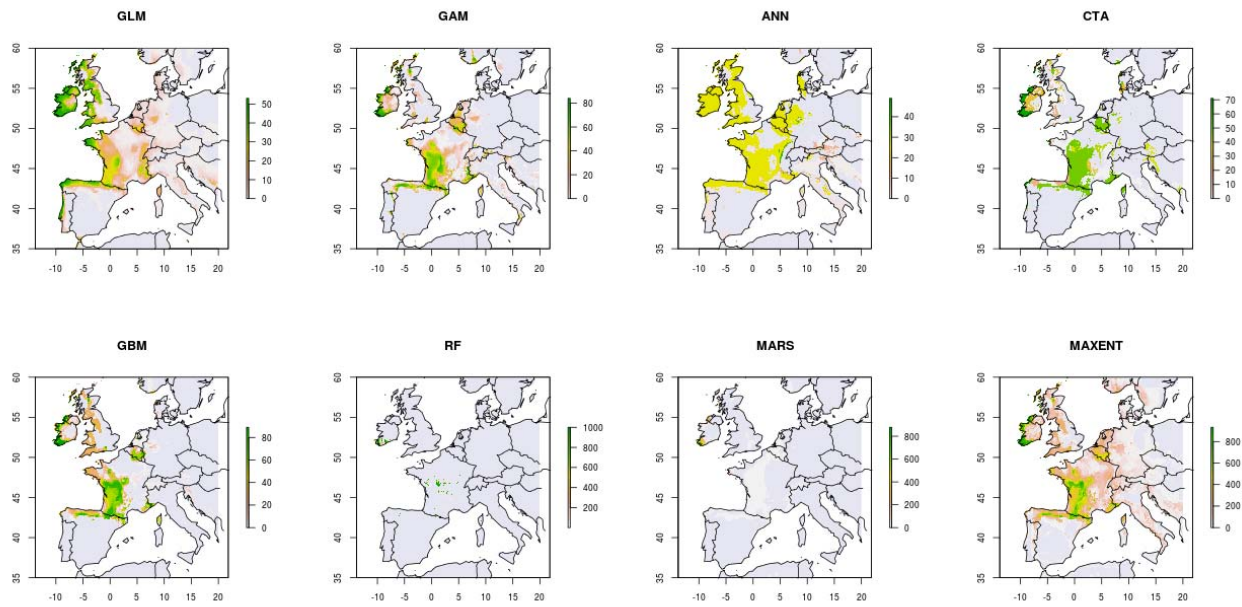


Figure S1. The eight model types used to generate the ensemble model showing the climate suitability for *Euphorbia hyberna* in the current climate. Here the suitability is shown with no range edge cut-off (compare with Fig.1 in the main text).

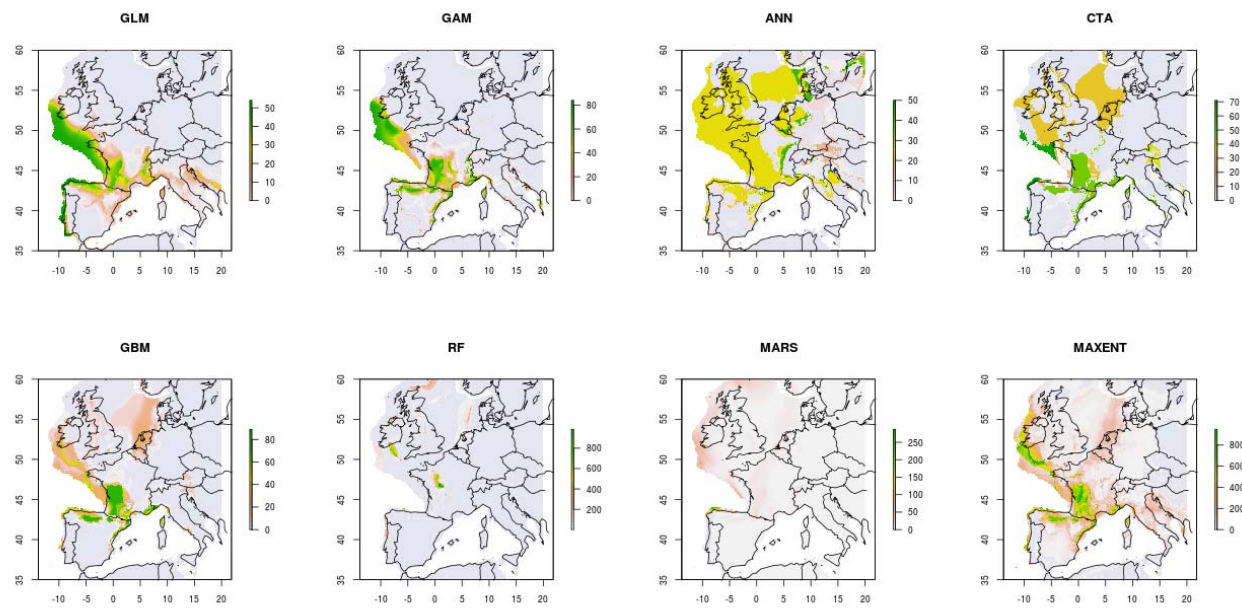


Figure S2. Similar to Fig. S1 but showing the modelled distribution of *Euphorbia hyberna* at the LGM.

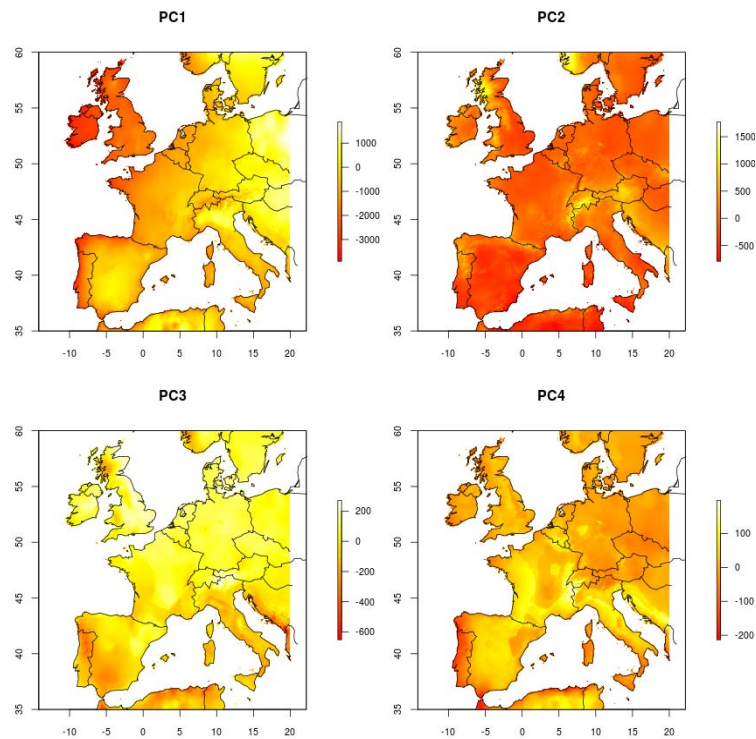


Figure S3. The four covariates used in the species distribution modelling for the current climate. These are the first four principal component scores of the 19 variable WorldClim dataset.

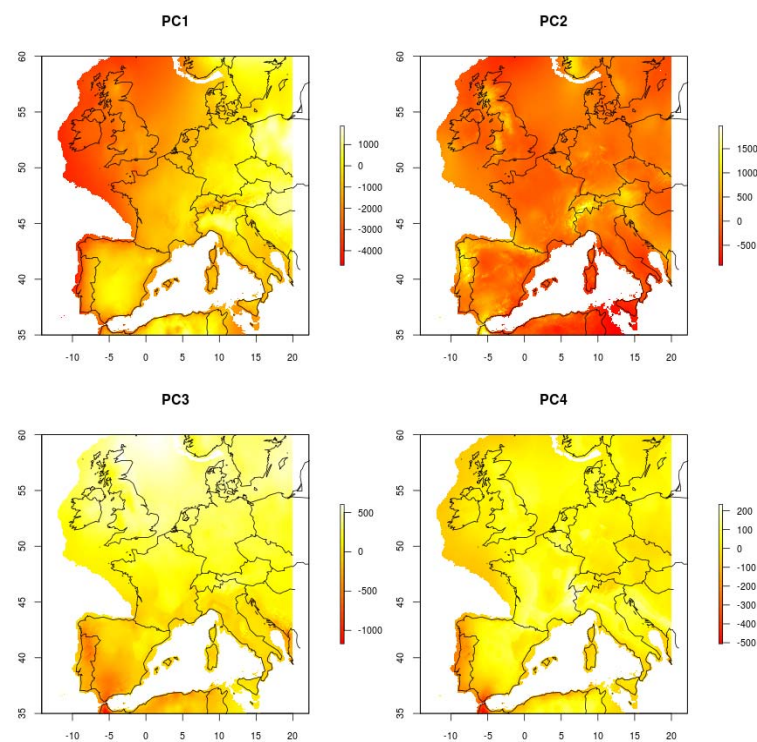


Figure S4. The four covariates used in the species distribution modelling for the LGM climate. These are PC scores based on the current climate PC but using the same variables from the CCSM climate dataset.

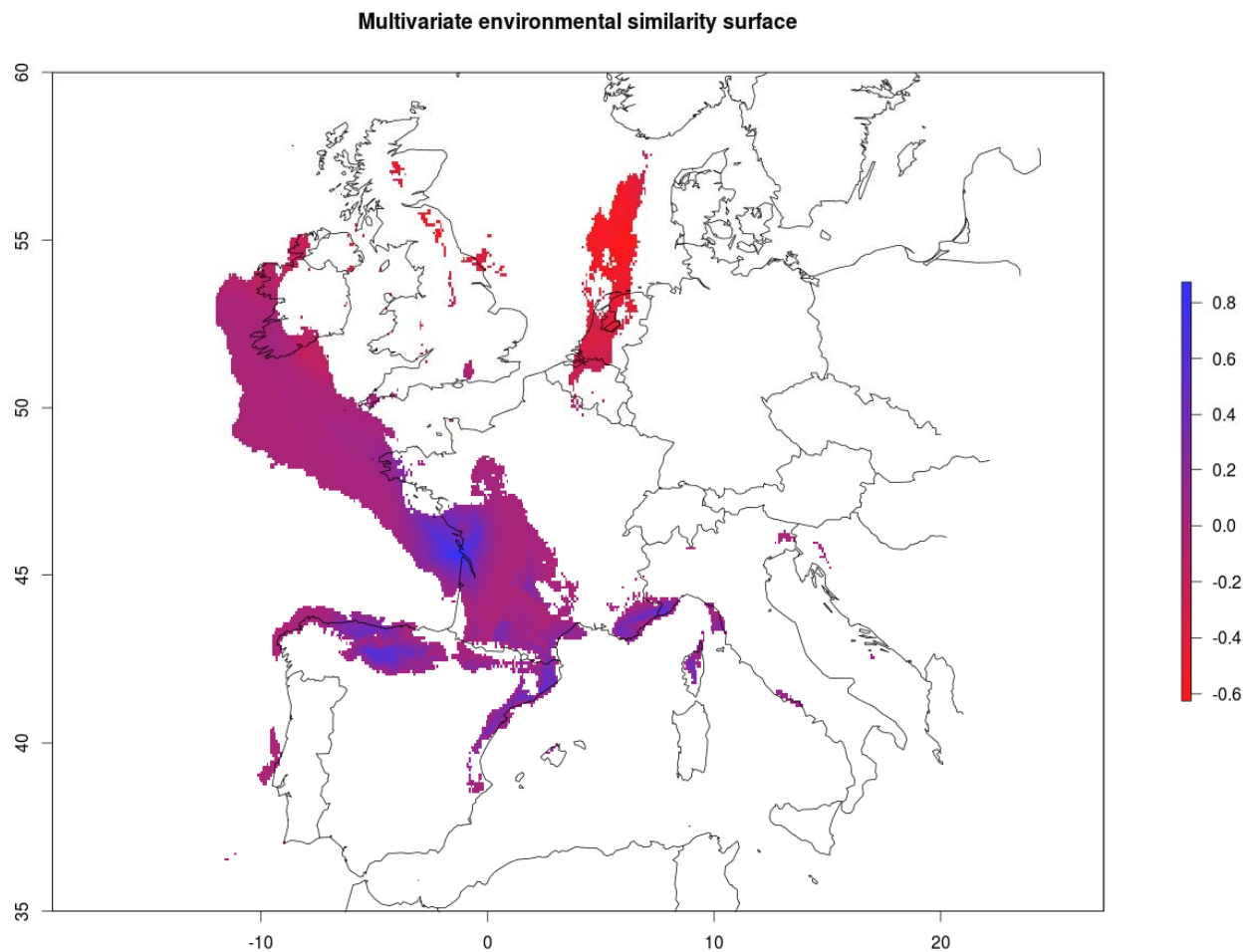


Figure S5. Multivariate environmental similarity surface (MESS) representing the similarity of the current climate, from which the species distribution models were constructed, to the LGM climate, for which they were projected. The colour scale represents similarity, such that low values (red) indicate a relatively large difference in climate while high values (blue) indicate small differences. Extrapolation into novel climate space is indicated by values below zero. Such areas only occur in the north, mainly where the ice sheet was present. The coloured area represents the projected distribution of *Euphorbia hyberna* at the LGM from the ensemble model. The MESS was calculated using the climate within the species range modelled in the current climate as the reference values compared with the climate within this LGM distribution (i.e. a comparison of climate within the two panels of Fig.2 in the main text).